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(21) International Application Number: PCT/US96/10507 (22) International Filing Date: 7 June 1996 (07.06.96) (30) Priority Data: 08/487,061 7 June 1995 (07.06.95) US (71) Applicant: INSTITUTE OF MATERIA MEDICA [CN/CN]; Xian Nong Tan Street 1, Beijing 100050 (CN). (71) Applicant (for SD only): MCINNIS, Patricia, A. [US/US]; 2324 42nd Street N.W., Washington, DC 20007 (US). (72) Inventors: HAN, Rui; Fan Cheng Yan Section I, Building 24, Beijing 100078 (CN). XU, Shi-Ping; Building 6, Nan Wei Road 2, Xuan Wu District, Beijing 100050 (CN). YUAN, Zhang-Liang; North Building 6, Yong An Road, Xuan Wu District, Beijing 100050 (CN). (74) Agents: BROWDY, Roger, L. et al.; Browdy and Neimark P.L.L.C., Suite 300, 419 Seventh Street, N.W., Washington, DC 20004 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: RETINOIDS AND METHODS OF USE OF SAME		
(57) Abstract New retinoid compounds, as coumarin retinoids and/or purine retinoids and compositions thereof, are provided which exhibit therapeutic and/or biological activity on cancer or precancer cells, as well as to methods of use of same.		

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RETINOIDS AND METHODS OF USE OF SAME

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

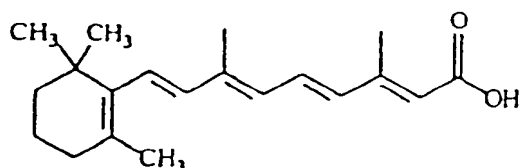
The present invention relates to novel retinoids, as coumarin retinoids and/or purine retinoids, and compositions thereof, which exhibit therapeutic and/or biological activity on cancer
10 or precancer cells, as well as to methods of using same.

DESCRIPTION OF THE RELATED BACKGROUND ART

Retinoids play an important role in the development and differentiation of epidermal cells, as well as in
15 reversing precancerous lesions. Clinical trials have been conducted using N-(4-carboxyphenyl) retinamide in the treatment of some precancerous lesions, oral leukoplakia, vulval leukoplakia, atypical dysplasia of the cervix and the gastric mucosa, and the like (Han et al., *in vivo* 4:153-160
20 (1990)). Other retinoids, such as isotretinoin and etretinate, are in current use as prescription drugs for the treatment of acne and psoriasis (Gander et al., U.S. Patent No. 4,126,693).

However, retinoids have a significant level of
25 toxicity, and treatment using known retinoid agents suffers from problems due to the level of toxicity and side effects which accompany administration of known retinoid compounds. Accordingly, there exists a need to provide novel compounds having retinoid activity but which have less toxicity and/or
30 side effects.

Smith et al., *J. Clin. Oncol.* 10 (5):839-864 (1992), reviews the use of retinoids in cancer therapy with most of the emphasis on anti-tumor effects in patients with acute promyelocytic leukemia (APL). Smith et al. reports that the
35 natural retinoid, all-trans-retinoic acid (RA),



40 induces cell differentiation in some human acute myeloid leukemia (AML), neuroblastoma, teratocarcinoma, melanoma, and

rat rhabdomyosarcoma cells and also appears to play an essential role in the normal differentiation of epithelial cells. Clinical toxicities associated with the use of RA in dermatologic studies are listed in Table 6 of the Smith et al. reference with disclosure in the text that retinoids are recognized as powerful teratogens.

Kizaki et al., *Seminars in Oncology* 19 (1) :95-105 (1992), reports that retinoids are potent anti-carcinogenic agents in many experimental models and that they inhibit growth and induce differentiation of transformed neoplastic cells. A number of references specifically relate to retinoic acid derivatives as anti-cancer agents. Just et al., U.S. Patent No. 4,385,175, discloses esters of retinoic acid with azetidiones useful as anti-cancer agents. Philippe et al., U.S. Patent No. 5,096,713, discloses retinoic esters of L cladinose which exhibit anti-tumor activity. The Paust Canadian Patent No. 1,127,170, is specifically directed to retinic acid N-(carboxy)-phenylamides and 7,8-dehydro-retinic acid N-(carboxy)-phenylamides, and discloses that, while the retinic compounds are predominantly directed toward preventing cancer, they may be used for therapeutic treatment of tumors of the bladder, the mammary gland, the skin and the mucous membranes.

A number of references disclose retinoids being used in cancer prophylaxis and as inducers of cell differentiation. For instance, Newton et al., *Cancer Res.* 40:3413-3425 (1980) discloses a long list of esters, amines and amides of retinoic acid and their activity in cancer prophylaxis. None of the listed compounds are related to flavonoids or chalcone retinoids. Du et al., *Inst. Mater. Med. Chinese Acad. Med. Sci. Beijing* 17:331 337 (1982), discloses the retinamides R_{II} (N-4(hydroxycarbophenyl) retinamide) and R_I (4-(ethoxycarbonyl phenyl)-retinamide) and their utility as cancer preventatives. Song et al., *Inst. Mater. Med. Chinese Acad. Med. Sci. Beijing* 19:576 581 (1984), also relates to the property of the retinamides R_I and R_{II} as cell differentiation inducers. The Shealy patent, U.S. Patent No. 5,124,083, discloses derivatives of retinoic acid as cell differentiation agents

for cancer prevention and treatment and the Shealy et al. publication, *J. Med. Chem.* 31:1124-1130 (1988), discloses the chemopreventative activity of bifunctional retinoic acid esters. Dawson, *J. Med. Chem.* 27:1516-1531 (1984), Dawson et al., *J. Med. Chem.* 24:583-592 (1981), Kagechika et al., *J. Med. Chem.* 32:1098-1108 (1989), Skrede et al., *Eur. J. Clin. Investig.* 21:574-579 (1991) and Loev et al., U.S. Patent No. 4,523,042, all disclose various retinoid compounds which are cell differentiation agents. Gander, U.S. Patent No. 4,323,581, discloses the use of N-(4-hydroxyphenyl)-all-trans retinamide for treatment of breast cancer while in U.S. Patent No. 4,310,546, Gander discloses the use of N-(4-acyloxyphenyl)-all-trans-retinamide in the prevention of epithelial cancer. The Gander et al. patents, U.S. Patent Nos. 4,126,693 and 4,190,594, relate to other properties of esters and amides of retinoic acids.

Furthermore, Blazeek et al., *Biomed. Pharmacother.* 45:169-177 (1991), Degos, *Biomed. Pharmacother.* 46:201-209 (1992), Castaigne et al., *Blood* 76 (9):1704-1709 (1990), Chomienne et al., *Blood* 76 (9):1710-1717 (1990), and Lo Coco et al., *Blood* 77 (8):1657-1659 (1991) all disclose using retinoic acid in differentiation therapy for patients with acute promyelocytic leukemia (APL).

Some flavonoids and chalcones have been found to have anti-tumor properties. Middleton et al., *Biochem. Pharmacol.* 43:1167-1179 (1992), relates to the anti-tumor effects of flavonoids, and Harvey et al., *J. Org. Chem.* 53:3936-3943 (1988) and *J. Org. Chem.* 55:6161-6166 (1990), and Nair et al., *Carcinogenesis* 12(1):65-69 (1991) relate to anti carcinogenic coumarin and flavone compounds. Jing et al., *Chinese J. Pharmacol. Toxicol.* 6 (4):278-280 (1992) discloses that an isoflavone, diadzein, inhibits melanoma cell growth. The Ishizuka et al. patent, U.S. Patent No. 5,096,924, discloses anti-cancer effects of a substituted 2 benzopyrinone. Ito et al., U.S. Patent No. 4,960,908, Briet et al., U.S. Patent Nos. 4,602,034, 4,783,533, and 5,116,954, and Kramer et al., U.S. Patent No. 4,713,465, all teach 4-benzopyrinone compounds as anti-cancer agents.

Preuss-Ueberschar et al., *Drug Res.* 34:1305-1313 (1984) discloses that benzopyrones, which include coumarin, are not teratogenic. The pharmacology of coumarin-related compounds is reviewed by Egan et al. which indicates that coumarin-related compounds are known to inhibit the carcinogenicity of carcinogens and that coumarin has been tested for treatment of melanoma.

Edwards et al., *J. Med. Chem.* 33:1948-1954 (1990) relates to the anti-mitotic action of chalcones. Cassady et al. and Ito et al., *Abstract Collection of International Symposium on Recent Advance in Chemistry and Molecular Biology of Cancer Research*, Beijing, China, 1991, pp. 5-6 and 68-69, respectively, relate to the anti-mitogenic effects of various flavonoids, including biochanin A.

Han et al., *Chinese J. Cancer Res.* 2 (3):51-53 (1990), reports the effects of an isoflavone, S86019, which is an active component of a medicinal herb used in China, *Pueraria lobata*. The structure of S86019 is not disclosed. In a different publication, *in vivo* 4:153-160 (1990), Han et al. evaluates the R_{II} retinamide compound as a cancer prevention agent and as a cancer chemotherapeutic agent and discusses the induction of cell differentiation of HL60 cells using a combination treatment of R_{II} with S86019. The combination of R_{II} with S86019 acts synergistically in inducing cell differentiation and it is reported that this combination may have clinical applications with minimal patient toxicity.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome one or more deficiencies of the related art, in particular, to overcome the teratogenicity associated with retinoids.

Another object of the present invention is to provide novel retinoids, as coumarin retinoids, and purine retinoids, which have anti-neoplastic activity *in vitro*.

A further object of the present invention is to
5 provide novel retinoids, as coumarin retinoids, or purine retinoids, which have anti-neoplastic activity *in vivo*.

A still further object of the present invention is to provide novel retinoid compounds and compositions, using methods of the present invention, which compounds and/or
10 compositions are useful for research and/or pharmacoutical applications in mammals, particularly humans.

The presently claimed retinoid compounds and compositions synergistically combine the cell differentiation activity of retinoids with the anti-carcinogenic properties of
15 coumarins.

One utility of the present invention is the use of such retinoids as a comparative compound or composition for *in vitro* testing of other compounds for anti-neoplastic activity. Such retinoids are also useful as chemotherapeutic agents in
20 *vitro*, *in situ* and/or *in vivo*.

Yet another object of the present invention is to provide synthetic methods for obtaining retinoid compounds and/or compositions according to formula (I) or (II), as descriptively enabled herein.

25- Furthermore, the invention is also directed to a method for treating a subject having a precancer or a cancer related pathology by administering at least one retinoid compound and/or composition comprising or consisting essentially of a coumarin retinoid and/or a purine retinoid,
30 optionally further comprising or consisting essentially of at least one anti-cancer pharmaceutical and/or immunomodulator.

Other features, advantages, embodiments, aspects and objects of the present invention will be clear to those skilled in the areas of relevant art, based on the
35 description, teaching and guidance presented herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the inhibitory effects of retinoids

on croton oil induced ODC activity in mouse epidermis ($\bar{x} \pm \text{SD}$, $n=3$).

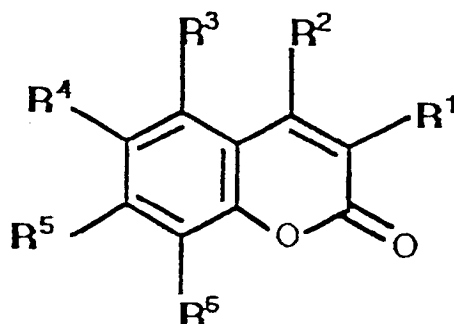
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DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides a new and biologically active group of retinoids which display cancer chemoprevention activity. These derivatives include, but are not limited to, compounds according to the following formula (I) as coumarin
10 retinoids or formula (II) as purine retinoids.

The coumarin-retinoids in accordance with the present invention have the following formula (I):

15



(I)

20

wherein $R^1 = \text{H}$, $\text{C}_1\text{-C}_{18}$ alkyl, or COR^7 , where $R^7 = \text{H}$, OH , $\text{C}_1\text{-C}_{18}$ alkoxy (preferably $\text{C}_1\text{-C}_2$ alkoxy), or NHR^8 , where $R^8 = \text{H}$, $\text{C}_1\text{-C}_{18}$ alkyl (preferably $\text{C}_1\text{-C}_2$ alkyl), or unsubstituted or substituted (in which the substituents are preferably selected from OH ,
25 COOH , OCH_3 , and NO_2) phenyl; R^2 is H , OH , $\text{C}_1\text{-C}_{18}$ alkyl (preferably methyl) or OR , where $\text{R} = \text{retinoyl}$; R^3 is H , CH_3 , COR^7 , OR , or CH_2OR , where R and R^7 are as defined above; R^4 is H , halogen (preferably Cl), $\text{C}_1\text{-C}_{18}$ alkyl, OH , OR , or COR^7 , where R and R^7 are as defined above; R^5 is H or OR , where R is as
30 defined above; and R^6 is H , CH_3 , OR or COR^7 , where R and R^7 are as defined above; and in which at least one of $\text{R}^2\text{-R}^6$ contains a retinoyl group.

The following compounds (I-1) - (I-38) shown in Table 1 are non-limiting examples of compounds in accordance
35 with the present invention according to Formula I where $\text{R} = \text{retinoyl}$:

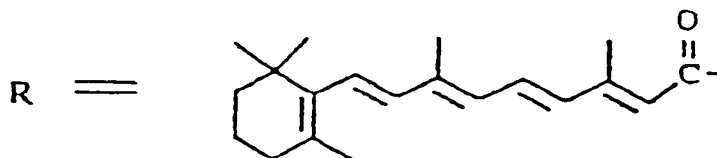
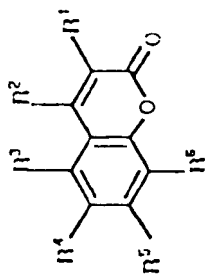


TABLE 1

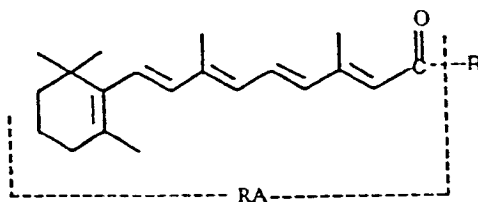


Compound	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶
I-1 SLM9133	COCH ₃	H	H	H	OR	H
I-2 XSP4-26	COCH ₃	H	CH ₃	H	OR	H
I-3 XSP4-15A	COCH ₃	H	H	H	OR	CH ₃
I-4 XSP4-12B	COCH ₃	H	CO ₂ H	H	OR	H
I-5 XSP5-5B	COCH ₃	H	H	H	OR	CO ₂ H
I-6 XSP4-13A	COCH ₃	H	H	Cl	OR	H
I-7 XSP4-21	COCH ₃	H	CH ₂ OR	H	OR	H
I-8 XSP5-4A	COCH ₃	H	OR	CO ₂ H	H	H
I-9 XSP5-4B	COCH ₃	H	H	H	OR	OR
I-10 SLM9123	COCH ₃	H	OR	H	OR	H
I-11 XSP4-22	COCH ₃	H	CO ₂ C ₂ H ₅	H	OR	H
I-12 SLM9134	CO ₂ C ₂ H ₅	H	CO ₂ C ₁₇ H ₂₅	H	OR	OR
I-13 XSP5-14B	COCH ₃	H	H	C ₂ H ₅	OR	H
I-14 XSP5-15A	COCH ₃	H	H	C ₆ H ₁₃	OR	CH ₃
I-15 SLM92-2	CO ₂ C ₂ H ₅	H	H	H	OR	CH ₃

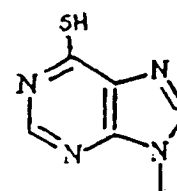
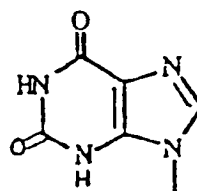
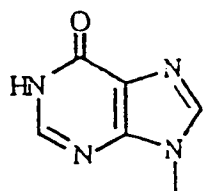
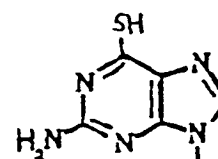
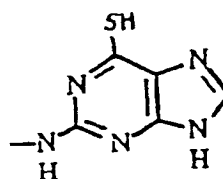
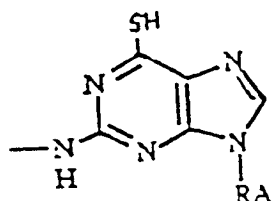
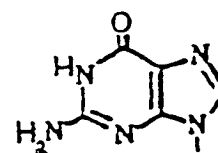
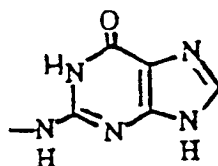
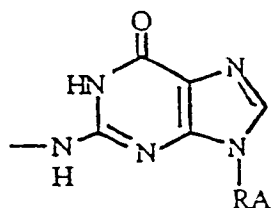
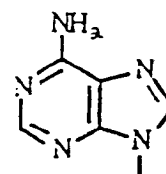
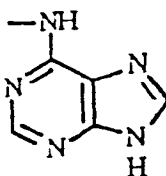
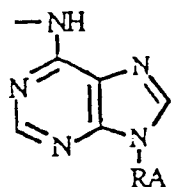
I-16 SLM _{III}	COCH ₃	H	H	COCH ₃	OR	H
I-17 XSP4-14A	CO ₂ C ₂ H ₅	H	H	H	OR	H
I-18 XSP4-30	CO ₂ C ₂ H ₅	H	H	Cl	OR	H
I-19 XSP4-25	CO ₂ C ₂ H ₅	H	CO ₂ H	H	OR	H
I-20 SLM9126	CO ₂ C ₂ H ₅	H	H	H	OR	OR
I-21 SLM9147	CO ₂ C ₂ H ₅	H	OR	H	OR	H
I-22 SLM _{III} -13	CO ₂ C ₂ H ₅	H	H	C ₂ H ₅	OR	H
I-23 XSP5-12B	CO ₂ C ₂ H ₅	H	H	H	OR	COCH ₃
I-24 SLM _{II} -20	CO ₂ C ₂ H ₅	H	OR	COC ₆ H ₅	H	H
I-25 SLM _{III} -12	CO ₂ H	H	H	C ₆ H ₁₃	OR	H
I-26 XSP5-5A	CONH ₂	H	CO ₂ H	H	OR	H
I-27 XSP5-3B	CONH ₂	H	H	Cl	OR	H
I-28 XSP5-29B	H	OR	H	t-Bu	H	H
I-29 XSP-30A	H	OR	H	H	H	CH ₃
I-30 XSP-30B	H	CH ₃	H	H	OR	CH ₃
I-31 S2-01B	H	CH ₃	H	C ₂ H ₅	OR	H
I-32 S41	H	CH ₃	H	H	OR	H
I-33 S42	H	CH ₃	OR	H	OR	H
I-34 S44	H	CH ₃	H	C ₆ H ₁₃	OR	H
I-35 S46A	H	OR	H	CH ₃	H	H
I-36 S46B	H	CH ₃	H	H	OR	OR
I-37	H	OR	H	H	CH ₃	H

I-38		H	OR	H	H	H	H	H
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The purine retinoids in accordance with the present invention have the following formula (II):



wherein R=



The foregoing compounds (II-A) - (II-L) provide non-limiting examples of compounds according to formula (II). Such compounds, or isomers thereof, in any combination are provided as compounds or compositions according to the present
5 invention.

Such retinoids of the present invention are unexpectedly discovered to have anti-cancer activity, thus providing suitable compounds and compositions for treatment of cancer-related pathologies, optionally with additional
10 pharmaceutically active ingredients, such as antiviral, chemotherapeutic agents and/or immuno-stimulating compounds, or antiviral antibodies or fragments thereof, *in vitro*, *in situ*, and/or *in vivo*.

Cancer-related pathologies in the context of the
15 present invention include, but are not limited to, tumors and pathologies involving tumorigenesis, leukemias, lymphomas, melanomas, sarcomas, virus-related cancers, and any other known cancers.

The term "anti-cancer activity" is intended to mean
20 the ability to induce at least one of (1) inhibition of growth or mitosis of transformed, mutated, preneoplastic or neoplastic cells; (2) promotion of apoptosis; and/or (3) angiogenesis inhibition.

In the context of the present invention, the term
25 inhibition or stimulation, as a quantitative value, is between 10 and 100 percent inhibition and 10 and 1000 percent stimulation, relative to a suitable control, such as, but not limited to the same cell or animal under the same conditions, except for the presence or administration of one or more
30 retinoids according to the present invention.

The present invention also provides synthetic methods for obtaining retinoid compounds according to formula (I) or formula (II), which would be clear to one of ordinary skill in the art, based on the teaching and guidance presented
35 herein, in combination with what is known in the related fields of art. In general, components of completely synthesized coumarin or purine compounds and/or retinoyl compounds can be provided as starting materials, and the

appropriate side groups can be added, modified or used in suitable, known chemical reaction steps to provide retinoid compounds according to formula (I) or formula (II).

5 Testing Anti-Cancer Activity

There are many known *in vitro* assays for determining whether a given compound and/or composition has antineoplastic activity. Such methods are well known in the art and provide the means for one of ordinary skill in the art to determine, using routine experimentation, whether a given retinoid of the present invention has a specific anti-cancer activity for a given cancer-related pathology.

The following are examples of methods which can be used to screen coumarin retinoids and purine retinoids according to formula (I) or formula (II) for determining at least one pharmaceutical utility; without undue experimentation, based on the teaching and guidance presented herein.

Non-limiting examples of anti-neoplastic *in vitro* activity, include but are not limited to, activity (1) against phosphorylation of phospholipids promoted by tetradecanoylphorbol-13-acetate in HeLa cells as a screening test for anti-tumor promoting effect; (see, e.g., Shibata, *Stem Cells* 12:44-52 (1994)); (2) soft agar clonogenic assays (e.g., Rangel et al., *Cancer Chemother. Pharmacol.* 33:460-64 (1994)); (3) cytostatic activity in cancer cells (see, e.g., Rajala et al., *Ann. Chir. Gynecol. Suppl.* 206:50-53 (1993)); inhibition of proliferation of human gastric cancer derived cells (Shibata, *supra*); (3) cytotoxic activity against cancer cell systems (see, e.g., Ngassapa et al., *J. Nat. Prod.* 15 56:1676-81 (1993); Sanyal et al., *Neoplasma* 40:219-22 (1993); Perez et al., *Cancer Chemother. Pharmacol.* 33:245-250 (1993)); Hahn et al., *Cancer* 72:2705-11 (1993)); (4) inhibition of tumor colony forming units (see, e.g., Eckardt et al., *J. Nat'l Cancer Inst.* 86:30-33 (1994); Chen et al., *Anticancer Drugs* 4:447-57 (1993)); (5) cell differentiation of human promyelocytic leukemia cells; and (6) MTT assay for inhibition of cancer cells, the contents of which references are entirely

incorporated herein by reference.

Non-limiting examples of anti-neoplastic *in vivo* activity include, but are not limited to, activity in (1) inhibition of tumorigenesis in mouse skin tumors (see, e.g., Shibata, *Stem Cells* 12:44-52 (1994)); (2) inhibition of animal carcinogenesis model systems (see, e.g., Kennedy, *Prev. Med.* 22:796-811 (1993); Johnson et al., *Cancer Chemother. Pharmacol.* 32:339-46 (1993)), such as nude mice or chimeric nude mice (see, e.g., Topp et al., *Blood* 82:2837-44 (1993); Sailkawa et al., *Jpn. J. Cancer Res.* 84:787-93 (1993)); (3) monocyte activation assay (Shi et al., *Cancer Res* 53:3986-91 (1993)); (4) serum tumor necrosis activity assay (Shi et al., *Cancer Res* 53:3986-91 (1993)); (5) croton oil induced ear edema in mice; and (6) croton oil induced ODC activity in mouse epidermis; the contents of which references are entirely incorporated herein by reference.

Additionally, predictive statistics and artificial intelligence can be used to provide computer programs which integrate matrix data to calculate patterns of activity, structural motifs, and a cell's expression of molecular targets to predict a given compound's mechanism of action to combine screening assay data and structure based drug design and testing, Weinstein et al., *Stem Cells* 12:12-22 (1994), the contents of which reference is entirely incorporated herein by reference.

Pharmaceutical Compositions. Pharmaceutical compositions comprising coumarin retinoid compounds and/or purine retinoid compounds of the present invention, include all compositions wherein at least one pharmaceutical compound or composition is contained in an amount effective to achieve its intended purpose. In addition, pharmaceutical compositions containing at least one pharmaceutical compound or composition may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the present invention can also include anti-neoplastics and/or immunomodulators.

A pharmaceutical compound or composition of the present invention may further comprise or consist essentially of at least one member selected from a single chain ribosome inhibitory protein (acting to block expression of a cancer related protein in a cancer related receptor cell or tissue);
5 a cytokine; or a growth factor.

Cytokines that are produced by lymphocytes are termed lymphokines, whereas peptides produced by monocytes or macrophages are given the term monokines. Thus, the terms
10 cytokines, lymphokines, and interleukins may be used interchangeably to designate those peptide molecules that modulate host responses to foreign antigens or host injury by regulating the growth, mobility and differentiation of leukocytes and other cells. Cytokines used according to the
15 present invention are those suitable for use as additional active ingredients of compositions of the present invention.

According to another aspect of the present invention, a cytotoxic or a chemotherapeutic agent may be further included in a pharmaceutical composition of the
20 present invention, optionally further comprising a delivery vector that preferentially binds to, or facilitates association of the pharmaceutical/diagnostic compound or composition with pathologic cells as target cells involved in cancer. The targets for this type of therapy can also be
25 growth factor receptors, differentiation antigens, or other less characterized cell surface antigens specifically associated with cancer or precancer cells.

Pharmaceutical compositions can also include suitable solutions for administration by injection or orally,
30 and contain from about 0.001 to 99 percent, preferably from about 20 to 75 percent of active component. Pharmaceutical compositions for oral administration include tablets and capsules. Compositions which can be administered rectally include suppositories.

35 Pharmaceutical carriers for the active ingredient may be either in sprayable or nonsprayable form. Nonsprayable forms can be semi-solid or solid forms comprising a carrier conducive to topical application and having a dynamic

viscosity preferably greater than that of water. Also suitable for systemic or topical application, in particular to the mucous membranes and lungs, are sprayable aerosol preparations where the active ingredient is preferably in combination with a solid or liquid inert carrier material. The aerosol preparations can contain solvents, buffers, surfactants, perfumes, and/or antioxidants in addition to the retinoid compounds or compositions of the present invention.

Pharmaceutical Administration. Pharmaceutical administration of a pharmaceutical compound or composition of the present invention may be administered by any means that achieve its intended purpose, for example, to treat or prevent a cancer or precancerous condition.

The term "protection", as in "protection from a cancerous or precancerous condition", as used herein, encompasses "prevention," "suppression" or "treatment." "Prevention" involves administration of a pharmaceutical composition prior to the induction of the disease. "Suppression" involves administration of the composition prior to the clinical appearance of the disease. "Treatment" involves administration of the protective composition after the appearance of the disease. It will be understood that in human and veterinary medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events may be unknown, latent, or not ascertained in the patient until well after the occurrence of the event or events. Therefore, it is common to use the term "prophylaxis" as distinct from "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis." See, e.g., Berkow et al, eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Goodman et al., eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, Inc., Elmeford, N.Y., (1990); Katzung, *Basic and Clinical Pharmacology*, Appleton and Lange, Norwalk, Conn., (1992), which are entirely incorporated herein by reference, including all references cited therein. The "protection" provided need

not be absolute, i.e., the disease need not be totally prevented or eradicated, provided that there is a statistically significant improvement ($p=0.05$) relative to a control population. Protection may be limited to mitigating the severity or rapidity of onset of symptoms of the disease.

At least one retinoid compound or composition of the present invention may be administered by any means that achieves the intended purpose, using a pharmaceutical composition as previously described.

For example, administration may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, intracranial, transdermal, or buccal routes. Parenteral administration can also be by bolus injection or by gradual perfusion over time. Alternatively, or concurrently, administration may be by the oral route.

An additional mode of using of a pharmaceutical compound or composition of the present invention is by topical application. A pharmaceutical compound or composition of the present invention may be incorporated into topically applied vehicles such as salves or ointments.

A typical regimen for treatment or prophylaxis includes administration of an effective amount over a period of one or several days, up to and including between one week and about six months.

It is understood that the dosage of a pharmaceutical compound or composition of the present invention administered *in vivo* will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the pharmacoutical effect desired. The ranges of effective doses provided herein are not intended to be limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one skilled in the relevant arts. See, e.g., Berkow et al, *supra*, Goodman et al, *supra*, and Katzung, *supra*; Avery's *Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and

Wilkins, Baltimore, MD. (1987), Ebadi, *Pharmacology*, Little, Brown and Co., Boston, (1985), which references are entirely incorporated herein by reference.

5 The total dose required for each treatment may be administered by multiple doses or in a single dose. The pharmacoutical compound or composition may be administered alone or in conjunction with other pharmaceuticals directed to the pathology, or directed to other symptoms of the pathology.

10 Effective amounts of a pharmaceutical compound or composition of the present invention are from about 0.001 μ g to about 100 mg/kg body weight, preferably about 5 mg/kg to 100 mg/kg body weight, most preferably about 20 mg/kg to 50 mg/kg body weight administered at intervals of 4-72 hours, for
15 a period of 2 days to 5 years.

The compounds and/or compositions of the present invention are to be administered to preferably mammalian recipients, most preferably humans.

20 Having now generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration, and is not intended to be limiting of the present invention.

Example 1: A Coumarin Retinoid Compound According to Formula I

25- Preparation of 3-acetyl-5-carboxy-7-retinoyloxy coumarin (I-4)

A 100 ml round-bottomed flask was fitted with a magnetic stirrer and a condenser, the upper end of which was protected by a calcium chloride tube. In the flask, a mixture of 0.6 g (0.002M) of retinoic acid, 20 ml of benzene and 0.12
30 ml (0.0013M) of PCl_3 was stirred for 1 hr at room temperature under N_2 . The reaction mixture was then concentrated with a rotavapor, and the residue was dissolved in 20 ml of absolute ether. This absolute ether solution was added dropwise at room temperature with stirring into a mixture of 0.5 g
35 (0.002M) of 3-acetyl-5-carboxy-7-hydroxycoumarin, 0.5 ml of pyridine and 20 ml absolute ether in a 100 ml, three necked, round-bottomed flask. After stirring for another two hrs., the solid produced was collected by filtration, then dried and

crystallized with ethanol. 0.7 g of the pure product was obtained with the following characteristics: mp: 145-47°C, NMR CDCl₃ δ 1.03(s, 6H, 1'-bis-CH₃); 1.38-2.14 (m, 6H, aliphatic cycle-CH₂-); 1.71(s, 3H, 5'-CH₃); 2.02(s, 3H, 9'-CH₃); 2.42(s, 3H, 13'-CH₃); 2.70(s, 3H, 3-COCH₃); 4.76(b, 1H, 5-CO₂H); 5.93-6.41 and 6.97-7.26, (m, 6H, double bond hydrogen of retinoyl); 7.40 (d, 1H, 8-H); 7.85(d, 1H, 6-H); 9.51 (s, 1H, 4-H); MS C₃₂H₃₄O₇=530 (M⁺), 282 (retinoyl), 248 (coumarin), 233 (100, 248-CH₃).

10

Example 2: A Coumarin Retinoid Compound According to Formula I

Preparation of 3-acetyl-5-ethoxycarbonyl-7-

retinoyloxy-coumarin (I-11) is under the same conditions as in Example 1 where 0.5 g of the product was obtained from 0.56 g

15 (0.002M) of 3-acetyl-5-ethoxycarboxy-7-coumarin and 0.6 g (0.002M) of retinoic acid (purified with ethanol), having mp: 162-64°C; NMR CDCl₃ δ 1.04(s, 6H-1'-bis-CH₃); 1.44(t, 3H, 5-ester-CH₃); 1.14-2.12(m, 6H, aliphatic cycle CH₂-); 1.72(s, 3H, 5'-CH₃); 2.03(s, 3H, 9'-CH₃); 2.42(s, 3H, 13'-CH₃); 2.70(s, 20 3H, 3-COCH₃); 4.24(q, 2H, 5-ester-CH₂); 5.88-6.48 and 6.98-7.25 (m, 6H, double bond hydrogen of retinoyl); 7.36 (d, 1H, 8-H); 7.73(d, 1H, 6-H); 9.47 (s, 1H, 4-H); MS C₃₄H₃₈O₇=558 (M⁺, nearly basic peak) 283, 276, 261, 233, 231, 175 (100, 231-28-28).

25 **Example 3: A Coumarin Retinoid Compound According to Formula I**

Preparation of 4-retinoyloxy-6-t-butyl-coumarin (I-

28) is under the same conditions as in Example 1 where 0.6 g of product was obtained (yield 43%) from 0.6 g of 4-hydroxy-6-t-butyl-coumarin and 1.0 g of retinoic acid, mp: 142-44°C.

¹HNMR δ (CDCl₃) 1.05(s, 6H, 1'-2CH₃), 1.37(s, 9H, t-But-3CH₃), 1.48, 1.62 and 2.04(m, 6H, cycle-CH), 1.73(s, 3H, 5'-CH₃), 2.05(s, 3H, 9'-CH₃), 2.46(s, 3H, 13'-CH₃), 6.06(s, 1H, 3-H), 35 6.16--6.52 and 7.16--7.26(m, 6H, alkene-H), 7.30(d, 1H, Ar-H), 7.61(m, 2H, Ar-H). MS:500(M⁺), 282, 218, 203, 175, 161.

Example 4: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-retinoyloxy-7-methyl-coumarin (I-37) is under the same conditions as in Example 1 where 0.5g of product was obtained (yield 50%) from 0.4 g of 4-hydroxy-7-methyl-coumarin and 1.0 g of retinoic acid, 115-117°C, ¹HNMR δ(CDCl₃) 1.03(s, 6H, 1'-2CH₃), 1.32--2.14(m, 6H, cycle--CH₂), 1.71(s, 3H, 5'-CH₃), 2.30(s, 3H, 9'-CH₃), 2.42(s, 6H, 7, 13'-2CH₃), 5.92(s, 1H, 3-H), 5.96--6.50 and 6.92--7.26(m, 6H, alkene-H), 7.10(m, 2H, Ar-H), 7.48(d, 1H, Ar-H). MS: 458(M⁺), 430, 355, 321, 307, 282, 267, 175, 159, 147.

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Example 5: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-methyl-7-retinoyloxy-coumarin (I-32) is under the same conditions as in Example 1 where 0.54 g of product was obtained (yield 39%) from 0.57 g of 4-methyl-7-hydroxy-coumarin and 1.0 g of retinoic acid, mp: 178--80°C, ¹HNMR δ(CDCl₃) 1.04(s, 6H, 1'-2CH₃), 1.16--2.28(m, 6H, cycle-CH₂), 1.73(s, 3H, 5'-CH₃), 2.01(s, 3H, 9'-CH₃), 2.28(s, 3H, 13'-CH₃), 2.41(s, 6H, 4, 8-2CH₃), 5.99(s, 1H, 3-H), 6.01--6.56 and 6.86--7.28(m, 6H, alkene-H), 7.06(d, 1H, ArH), 7.40(d, 1H, Ar-H). MS: 472(M⁺), 283, 190, 175, 161.

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Example 6: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-methyl-6-ethyl-7-retinoyloxy-coumarin (I-31) is under the same conditions as in Example 1 where 0.43 g of product was obtained (yield 43%) from 0.61 g of 4-methyl-6-ethyl-7-hydroxy-coumarin and 1.0 g of retinoic acid, mp: 120-2°C, ¹HMR δ(CDCl₃) 1.05(s, 6H, 1'-2CH₃), 1.22(t, 3H, 6-Et-CH₃), 1.16-2.24(m, 6H, cycle-CH₂) 1.72(s, 3H, 5'-CH₃), 2.02(s, 3H, 9'-CH₃), 2.41(s, 3H, 13'-CH₃), 2.66(q, 2H, 6-Et-CH₂), 6.00(s, 1H, 3-H), 6.06--6.50 and 6.88-7.26(m, 6H, alkene-H), 7.07(s, 1H, Ar-H), 7.41(s, 1H, Ar-H). MS: 486(M⁺), 470, 363, 283, 204, 189, 175, 161.

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Example 7: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-methyl-7-retinoyloxy-coumarin is under the same conditions as in Example 1 where 0.64 g was obtained (yield 61.5%) from 0.4 g of methyl-7-hydroxy-coumarin

35

and

0.9 g of retinoic acid, mp: 154--6°C, ¹HMR, δ(CDCl₃): 1.04(s, 6H, 1'-2CH₃), 1.47, 1.62 and 1.94(m, 6H, cycle-CH₂), 1.72(s, 3H, 5'-CH₃), 2.03(s, 3H, 9'-CH₃), 2.42--2.44(d, 6H, 4, 13'-2CH₃), 5.98(s, 1H, 3-H), 6.15--6.39 and 7.09--7.12(m, 6H, alkene-H), 7.15(m, 2H, Ar-H), 7.60(d, 1H, Ar-H). MS: 458(M⁺), 283, 175, 161, 148.

Example 8: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-methyl-5,7-diretinoyloxy-coumarin (I-33) is under the same conditions as in Example 1 where 0.75 g was obtained (yield 36.6%) from 0.52 g of 4-methyl 5,7-dihydroxy-coumarin and 2 g of retinoic acid, mp: 134--36°C, ¹HMR, δ(CDCl₃): 1.04(s, 12H, 1', 1''-4CH₃), 1.48, 1.62 and 2.00[m, 12H, bis-(cycle-CH₂)], 1.73(s, 6H, 5', 5''-2CH₃), 2.04(6H, 9', 9''-2CH₃), 2.48(d, 6H, 13', 13''-2CH₃), 2.63(s, 3H, 4-CH₃), 5.97(s, 1H, 3-H), 6.15--6.40 and 6.93--7.17(m, 12H, alkene-H), 7.13(d, 1H, Ar-H), 7.16(d, 1H, Ar-H). MS: 474(M⁺ 282), 283, 192, 175, 161.

Example 9: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-methyl-6-hexyl-7-retinoyloxy-coumarin (I-34) is under the same conditions as in Example 1 where 1.0 g was obtained (yield 41.0%) from 0.7 g of 4-methyl-6-hexyl-7-hydroxy-coumarin and 1.0 g of retinoic acid, mp: 114--5°C. ¹HMR, δ(CDCl₃): 0.87(t, 3H, 6-hex-CH₃), 1.04(s, 6H, 1'-2CH₃) 1.02--2.63(m, 14H, 6-hex- and cycle-CH₂) 1.73(s, 3H, 5'-CH₃), 2.03(s, 3H, 9'-CH₃), 2.42(s, 6H, 4, 13'-2CH₃), 2.58(m, 2H, 6-hex-CH₂), 6.02(s, 1H, 3-H), 6.16--6.41 and 7.06--7.40(m, 6H, alkene-H), 7.10(s, 1H, Ar-H), 7.42(s, 1H, Ar-H). MS: 542(M⁺), 527, 283, 260, 189, 175, 161, 135.

Example 10: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-retinoyloxy-6-methyl-coumarin (I-35) is under the same conditions as in Example 1 where 0.73 g was obtained (yield 56.3%) from 0.5 g of 4-hydroxy-6-methyl-coumarin and 1.0 g of retinoic acid, mp: 104--6°C, ¹HMR,

δ (CDCl₃): 1.04 (s, 6H, 1'-2CH₃), 1.42, 1.62 and 2.03 (m, 6H, cycle-CH₂), 1.73 (s, 3H, 5'-CH₃), 2.04 (s, 3H, 9'-CH₃), 2.43 (d, 6H, 6, 13'-2CH₃), 6.05 (s, 1H, 3-H), 6.17--6.50 and 7.16 (m, 6H, alkene-H), 7.25 (m, 2H, Ar-H), 7.37 (d, 1H, Ar-H). MS: 458 (M⁺), 444, 430, 282, 176, 161.

Example 11: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-methyl-7,8-diretinoyloxy-coumarin (I-36) is under the same conditions as in Example 1 where 1.65 g was obtained (yield 73.5%) from 0.57 g of 4-Methyl-7,8-dihydroxy-coumarin and 1 g of retinoic acid, mp: 106--8°C. ¹HNMR, δ (CDCl₃), 1.03 (s, 12H, 1', 1''-4CH₃), 1.10--2.61 (m, 12H, cycle-CH₂), 1.66 (s, 6H, 5', 5''-2CH₃), 2.00 (s, 6H, 9', 9''-2CH₃), 2.17 (s, 6H, 13', 13''-2CH₃), 2.24 (s, 3H, 4-CH₃), 5.92 (s, 1H, 3-H), 6.04--6.91 and 6.84--7.14 (m, 12H, alkene-H), 7.18 (d, 1H, Ar-H), 7.44 (d, 1H, Ar-H). MS: 474 (M⁺), 282, 283, 192, 175, 161.

Example 12: A Coumarin Retinoid Compound According to Formula I

Preparation of 4-retinoyloxy-coumarin (I-38) is under the same conditions as in Example 1 where 0.65 g was obtained (yield 30%) from 0.8 g of 4-hydroxy-coumarin and 2.0 g of retinoic acid. mp: 88-91°C. ¹HNMR δ (CDCl₃), 1.04 (s, 6H, 1'-CH₃), 1.48--2.62 (m, 6H, cycle-CH₂), 1.64 (s, 3H, 5'-CH₃), 2.04 (s, 3H, 9'-CH₃), 2.36 (s, 3H, 13'-CH₃), 6.03 (s, 1H, 3-H), 6.13--6.5 and 7.01--7.21 (m, 6H, alkene-H), 7.27--7.69 (m, 4H, Ar-H). MS: 444 (M⁺), 429, 416, 282, 255, 175, 161, 159, 135.

Example 13: Preparation of a purine retinoid according to formula (II)

For the preparation of the desired compounds, all-trans-retinoic acid (0.01 M) and phosphorus trichloride (0.003 M) were combined in a round bottom flask and 60 ml petroleum ether was added and stirred. The reaction mixture in the flask was heated with a rotavapor. At this step, hydrogen chloride was produced and allowed to escape with the reaction

solution turning to be brown-yellowish transparent solution. The reaction solution was decanted and distilled to remove the solvent and the residue was then dissolved with acetate ether.

Compounds with an amino or hydroxy group (0.01 M) was dissolved in an appropriate solvent at room temperature and the prepared retinoyl chloride was added dropwise. The resultant precipitate was formed instantly and the color of reaction solution deepened. The reaction was allowed to continue for two hours or overnight at room temperature. The reactant was filtered and the resultant product was washed or recrystallized. The filtrate was concentrated and recrystallized or chromatographed for purification. The product was checked by thin layer chromatography and the melting point of the compound was also studied. The parameters for structure identification of purine retinoid II-A were as follows:

UV: λ_{max} - EtOH 340 nm E = 5180

IR: KBr cm^{-1}
1690 -CONHR, 1670 -CON<

HNMR: CDC13 δ ppm

1.05 (12H, S, CH₃) 1.5 ~ 1.7 (8H, m, CH₂) 1.75 (6H, S, CH₃)

2.05 (6H, S, CH₃) 2.55 (6H, S, CH₃) 2.05 (4H, t, CH₂)

Element Analysis:

calculated value:	C 75.28, H 8.28, N 9.75
determined value:	C 75.27, H 8.55, N 9.33

40 **Example 14 Cell Differentiation Activity of Coumarin Retinoids**

Human promyelocytic leukemia HL-60 cells were cultured in RPMI-1640 medium supplemented by 10% heat-inactivated calf serum and 100 U/ml of penicillin plus 100 μ g/ml of streptomycin. The flasks containing cells and medium were incubated in an incubator with 5% CO₂ at 37°C. Log phase

cells were seeded into the medium at a concentration of 1.2×10^5 to 1.4×10^5 cells per ml and cultured in flasks containing 5 ml of medium. Each group consisted of three flasks. Different concentrations of coumarin-retinoids were added. At intervals following the addition of coumarin-retinoids to the cultures, a small portion of cells was removed and live cells were counted under a light microscope using a dye-exclusion method with trypan blue. Cell differentiation was judged in terms of morphology and nitroblue tetrazolium (NBT) reduction.

For a determination of NBT reducing activity, a portion of the coumarin-retinoid-treated cells was removed at intervals following treatment and centrifuged. After centrifugation, 0.5 ml of 0.1% NBT (containing 100 ng TPA) was added to each tube. The contents of the test tubes were then incubated for 1 hour at 37°C. Cell smears were prepared from the cell sediment and stained using the Wright-Giemsa technique. For each smear prepared, 200 cells were isolated for examination under a light microscope with an oil immersion lens. Cells stained with blue-black granules were considered NBT-positive cells. The 50% differentiation or effective concentration (ED_{50} or EC_{50}) is shown in terms of molar (M) concentration of the coumarin-retinoid tested.

For a determination of cell morphology, a cell smear was prepared at intervals following treatment of cells for each coumarin-retinoid concentration group using the Wright-Giemsa stain technique. Stained cells were counted and classified under a light microscope with an oil immersion lens. After treatment with coumarin-retinoids, the morphological development of HL-60 cells proceeded toward mature granulocytes. This development was manifested by decreased cell volume; a lower nuclei to cytoplasm ration; smaller or missing nuclei; concentration of chromatin; and the appearance of a certain proportion of intermediate and late granuloblasts (progranulocytes), elongated rod granulocytes, and granulocytes with branched nuclei.

TABLE 2

Compound	Concentration (M)	NBT(+) %	EC ₅₀ (M)
I-11	10 ⁻⁴	77	3x10 ⁻⁶
	10 ⁻⁵	72	
	10 ⁻⁶	49	
	10 ⁻⁷	8	
5 I-19	10 ⁻⁴	92	9x10 ⁻⁶
	10 ⁻⁵	84	
	10 ⁻⁶	5	
I-2	10 ⁻⁵	84	2x10 ⁻⁶
	10 ⁻⁶	38	
	10 ⁻⁷	2	
I-12	10 ⁻⁵	82	1x10 ⁻⁸
	10 ⁻⁶	47	
	10 ⁻⁷	30	
	10 ⁻⁸	0	
	10 ⁻⁹	0	
I-10	6.4x10 ⁻⁶	99	1.3x10 ⁻⁸
	6.4x10 ⁻⁷	96	
	6.4x10 ⁻⁸	82	
	6.4x10 ⁻⁹	33	
	6.4x10 ⁻¹⁰	10	

Compound	Concentration (M)	NBT(+) %	EC ₅₀ (M)
I-6	10 ⁻⁴	96	6x10 ⁻⁷
	10 ⁻⁵	81	
	10 ⁻⁶	78	
	10 ⁻⁷	31	
	10 ⁻⁸	2	
I-7	10 ⁻⁵	85	7.5x10 ⁻⁷
	10 ⁻⁶	65	
	10 ⁻⁷	12	
I-27	10 ⁻⁵	88	5.5x10 ⁻⁷
	10 ⁻⁶	80	
	10 ⁻⁷	17	
I-8	10 ⁻⁵	98	5.9x10 ⁻⁸
	10 ⁻⁶	69	
	10 ⁻⁷	55	
	10 ⁻⁸	34	
I-9	10 ⁻⁵	96	6.5x10 ⁻⁸
	10 ⁻⁶	88	
	10 ⁻⁷	55	
	10 ⁻⁸	31	
I-15	10 ⁻⁵	93	2.6x10 ⁻⁷
	10 ⁻⁶	84	
	10 ⁻⁷	46	
	10 ⁻⁸	12	

Compound	Concentration (M)	NBT(+) %	EC ₅₀ (M)
I-21	10 ⁻⁵	94	2.5x10 ⁻⁷
	10 ⁻⁶	80	
	10 ⁻⁷	41	
	10 ⁻⁸	17	
I-26	10 ⁻⁵	97	8.0x10 ⁻⁸
	10 ⁻⁶	91	
	10 ⁻⁷	53	
	10 ⁻⁸	34	
I-5	10 ⁻⁵	89	2.8x10 ⁻⁷
	10 ⁻⁴	69	
	10 ⁻⁷	41	
I-4	10 ⁻⁵	92	7x10 ⁻⁸
	10 ⁻⁶	84	
	10 ⁻⁷	73	
	10 ⁻⁸	15	
I-18	10 ⁻⁵	44	1x10 ⁻⁵
	10 ⁻⁶	8	
I-17	10 ⁻⁵	84	1x10 ⁻⁶
	10 ⁻⁶	54	
	10 ⁻⁷	2	
I-3	10 ⁻⁵	88	8x10 ⁻⁷
	10 ⁻⁶	77	
	10 ⁻⁷	12	

Example 15: Anti-Teratogenicity Effect of Retinoids on Fetus Absorption in Mice

Female mice were examined by vaginal smear the morning after being caged with male mice, and if sperm or a vaginal plug was discovered, then the mice were determined to be pregnant. The retinoids were administered at the dosage indicated starting on the seventh day of pregnancy and continued once a day for the next ten days. On the 20th day after the female mice were determined to be pregnant, they were sacrificed by cervical dislocation. Both sides of the uterus were checked and the number of living embryos, dead embryos, and absorbed embryos were counted. The living embryos were further examined for bone abnormalities.

TABLE 3

Effect of I-10 on fetus absorption in mice

Group	Dosage (mg/kg)	Fetus Absorption (%)
		absorbed number/implanted number
Control		0/135
Tween 80	0.3%	0/104
I-10	40	0/107
	20	0/104
All Trans RA	10	73/99 (73.7%)

TABLE 4

Effect of I-9 on fetus absorption in mice

Group	Dosage (mg/kg)	Fetus Absorption (%)
		absorbed number/implanted number
Control		0/135
Tween 80	0.3%	0/104
I-9	40	0/119
	20	0/113
All Trans RA	10	73/99 (73.7%)

TABLE 5

Effect of II-A on fetus absorption in mice

Group	Dosage (mg/kg)	Pregnant Mice	Implanted Number	Absorbed Number
Control		14	153	0
II-A	20	12	141	10
	40	15	181	17
Tween 80	0.3%	15	184	4
All Trans RA	15	15	186	111

Example 16: Effect of Coumarin Retinoids on Croton Oil Induced Ornithine Decarboxylase Enzyme Activity in Mouse Epidermis

5 ICR mice (6-8 weeks old) were pretreated with a single dose of coumarin retinoid. On the third day, the animals received a topical application of 0.2 ml of 1% croton oil in acetone. All animals were sacrificed five hours after the croton oil treatment and the epidermis was separated by a
10 brief heat treatment at 52°C for 30 seconds and minced in a blender. The cell suspension and broth were separated by a centrifugation at 30,000xg for 30 seconds and the supernatant was obtained. Ornithine decarboxylase (ODC) enzyme activity was determined by measuring the release of CO₂ from L-(¹⁴C)-
15 ornithine and expressed as nmole CO₂/30 min/mg protein and the results for coumarin retinoid I-10 and I-6 are shown in Figure 1. As can be seen in Figure 1, the coumarin retinoids significantly inhibited the ODC activity induced by croton oil. ODC activity is a critical parameter for monitoring
20 cancer chemoprevention or promotion.

Example 17: Anti-Oxidation Effect of Coumarin Retinoids

Lipid peroxidation was induced by adding 1 mM FeSO₄ and 10 mM cysteine to the microsome fraction of rat liver with
25 the incubations being carried out at 37°C for 30 minutes in a reaction mixture containing 10 mM phosphate buffer (pH 7.4). The reaction was terminated by the addition of 0.3 ml of 20% trichloroacetic acid solution. Thiobarbituric acid was then added and the mixture was boiled for 10 minutes. After
30 cooling the test tubes under running tap water, the optical density at 532 nm was measured. Lipid peroxidation in both the treated groups and the control group was expressed in nmol MDA/mg protein. The percentage inhibition indicates the anti-oxidation properties of the coumarin retinoids tested.

35 **Example 18: Anti-Mutagenicity Effect of Coumarin Retinoids**

An antimutagenicity assay was conducted according to the method developed by Ames et al. using *Salmonella*
40 *typhimurium* strains TA 102 and TA 100 and mammalian microsomal

enzymes. Beta-naphthoflavone and phenobarbital-induced rat liver supernatant (9000xg S-9 fraction) was used as the enzyme source for metabolic activation. The night before the experiment, certified bacterial solution (assessed for its biological characteristics and donated by Professor Ames) was inoculated into petri dishes. The petri dishes were then incubated for 15 hours at 37°C. From each petri dish, 0.1 ml of the bacterial solution was removed and added to sterile test tubes. To these test tubes, 0.1 ml of different concentrations of coumarin retinoids and 0.5 ml of either S-9 mixed solution or phosphate buffer were then added. The final solution for each test tube was incubated in a water bath at 37°C for 20 minutes before adding 2 ml of agar solution. After thorough mixing, the solution was transferred into petri dishes and incubated once again at 37°C for 48 hours. After incubation, the number of colonies in each petri dish were counted. The revertant numbers of the bacteria in control and treated groups were compared and the inhibition rate was calculated.

TABLE 6

Inhibition of I-6 and I-5 on Fe²⁺/Cys induced lipid peroxidation of rat liver microsome (n=3)

Group	Concentration (μM)	Lipid Peroxidation (μM MDA/mg protein)	Inhibition (%)
Control		2.38 ± 0.12	
I-6	2	2.16 ± 0.18	8.6
	20	2.13 ± 0.19	9.6
	200	2.03 ± 0.16*	13.5
I-5	1	2.19 ± 0.13	7.3
	10	1.95 ± 0.18*	16.6
	100	1.43 ± 0.16**	36.6

*p < 0.05

**p < 0.01

TABLE 7

Effect of I-6 on the induction of His⁺/revertants by MMS mutagen (0.5 μ l/plate) in *Salmonella typhimurium* strains TA100 and TA102 (n=3)

Group	Concentration (mg/plate)	his ⁺ -revertants/per plate		
		TA100	Inhibition (%)	TA102
MMS		1083.7 \pm 99.3		1760 \pm 288.4
I-6	0.1	971.3 \pm 102.7	12.8	1560 \pm 40.0
	0.5	947.3 \pm 60.3	15.5	1375 \pm 241.5
				Inhibition (%)
				26.6

Example 19: Effect of Coumarin Retinoids on Croton Oil Induced Ear Edema in Mice

ICR mice (body weight 18-20 grams) were used. Each group consisted of 10 mice. For the treated group, coumarin retinoids were administered at the dosage indicated in the table for 10 days. After three dosage treatments, the left ears of the mice in both the treated group and the control group were painted with 2% croton oil in 0.05 ml ether. All the mice were sacrificed six hours later and the ears of both sides were punched by a corneal knife and weighed, the difference of the ear weight of both sides for each mouse was calculated. The t- Student test was used for statistical calculation. Croton oil induced ear edema is a classical assay for evaluation of cancer promotion.

TABLE 8

Inhibition of I-10 and I-6 on
croton oil induced ear edema in mice

Groups	Dose (mg/kg)	Ear Edema ($\bar{x} \pm SD$) mg	IR (%)	P
Control		16.5 \pm 1.4		
I-10	50	14.2 \pm 2.3	14	< 0.05
	100	11.3 \pm 3.0	32	< 0.01
I-6	50	13.4 \pm 3.1	19	< 0.01
	100	15.1 \pm 2.7	9	

Example 20: Anti-Teratogenicity Effect of Retinoids on Bone Abnormalities in Mouse Fetus

Living embryos, removed from female mice as described in Example 15, were placed in 95% ethanol until fixed, and then placed in an alizarin dye and dyed red. After the embryos became transparent, they were dissected and their skeletons were checked by observation under a microscope.

TABLE 9

Effect of I-10 on bone abnormalities in mouse fetus

Group	Dosage (mg/kg)	Delayed Calcification (%)	
		Occipital delay/fetus	Sternum delay/fetus
Control		0/79	0/79
Tween 80	0.3%	0/69	0/69
I-10	40	15/81	21/81
	20	22/66	14/66
All-trans RA	101	6/16	16/16

TABLE 10

Effect of I-9 on bone abnormalities in mouse fetus

Group	Dosage (mg/kg)	Delayed Calcification (%)	
		Occipital number/fetus number	Sternum number/fetus number
Control	--	0/79	0/79
Tween 80	0.3%	0/69	0/69
I-9	40	3/77	2/77
	20	0/75	0/75
All-trans RA	10	16/16	16/16

TABLE 11

Effect of II-A on Bone Abnormalities in Mouse Fetus

Group	Dosage (mg/kg)	Delayed Calcification (%)		Cleft Palate (%)
		Occipital	Sternum	
Control	-	0	0	0
Solvent	-	5.4	5.4	0
II-A	20	7.6	9.0	0
	40	7.2	7.2	0
Retinoic Acid	15	96.0	71.0	48

Example 21: Inhibitory Effect of Coumarin Retinoids on Cancer Cells

Human cancer cell lines (KB, A2780, MCF-7, HL-60, A549) were used. For a monolayer culture, 0.5 ml of trypsin solution (0.3 gm/ml) was added to the flasks for digestion. Microtiter plates (96 well) were used for the cell culture and 200 μ l of cell suspension (1200 tumor cells) were placed in each well and incubated at 37°C for 24 hours. Following the addition of the test compound, the control and treated flasks were placed in a CO₂ incubator for 5 day culture. For the MTT assay, 200 μ l of MTT solution (5 mg in 10 ml of phosphate buffer) plus the medium were added and incubated for another 4 hours. Following incubation, the supernatant was discarded and 200 μ l of DMSO was added to each well. With a mild agitation the OD values were determined by the MR 700 Elisa at 570 nm (reference wavelength was 450 nm). The ED₅₀ of the compound and tumor cell survival rate

$$\text{Tumor cell survival} = \frac{\text{OD of treated group}}{\text{OD of control group}}$$

as percent inhibition were calculated.

TABLE 12

Results on MTT Assay in HL-60 Cells

Compound	Concentration (M)	Inhibition %	ED ₅₀ (M)
I-8	10 ⁻⁵	63.9±2.9	1x10 ⁻⁷
	10 ⁻⁶	54±1.9	
	10 ⁻⁷	50.5±0.9	
	10 ⁻⁸	29.7±1.4	
I-9	10 ⁻⁵	61.5±1.9	9.5x10 ⁻⁷
	10 ⁻⁶	50.6±0.5	
	10 ⁻⁷	48.8±0.5	
	10 ⁻⁸	28.0±5.2	
I-15	10 ⁻⁵	54.5±2.8	9x10 ⁻⁷
	10 ⁻⁶	48.8±1.3	
	10 ⁻⁷	34.6±0.0	
	10 ⁻⁸	16.2±2.4	
I-21	10 ⁻⁵	61.2±2.4	1x10 ⁻⁶
	10 ⁻⁶	49.9±2.5	
	10 ⁻⁷	32.5±1.1	
	10 ⁻⁸	21.8±3.1	
I-26	10 ⁻⁵	68.7±0.2	9x10 ⁻⁷
	10 ⁻⁶	52.6±0.5	
	10 ⁻⁷	43.8±1.3	
	10 ⁻⁸	9.4±3.7	

Compound	Concentration (M)	Inhibition %	ED ₅₀ (M)
I-5	10 ⁻⁵	51.6±0.0	8.5×10 ⁻⁶
	10 ⁻⁶	34.0±7.1	
	10 ⁻⁷	11.5±4.2	
I-13	10 ⁻⁵	5.5±3.9	>10 ⁻⁵
	10 ⁻⁶	4.3±6.1	
	10 ⁻⁷	3.5±3.9	
I-14	10 ⁻⁵	52.6±1.3	9.0×10 ⁻⁶
	10 ⁻⁶	30.0±1.4	
	10 ⁻⁷	10.4±2.4	

**Example 22: Effect of Retinoids on Growth of Chondrosarcomas
in Rats**

Transplantable chondrosarcomas obtained from Dr.

5 Trown, Hoffman - La Roche Institute of Molecular Biology and
Wistar rats obtained from the Breeding Center of Experimental
Animals, Chinese Academy of Medical Sciences were used.
According to the routine transplantation protocol as described
in *Cancer Treatment Reports* 60(11):1647-1653 (1976) where the
10 small pieces of chondrosarcoma tumor were implanted
subcutaneously in the right inguinal region of rats using a
trocar. Tumor-bearing rats were randomized to a control group
and a treated group. Each group consisted of ten rats. The
compound was given orally for 12 days at the indicated dosage.
15 Sixty days after transplantation, the rats were sacrificed and
the tumor weight was determined. The inhibition rate was
calculated according to the following formula:

$$\text{Inhibition rate} = \frac{\text{tumor weight of control} - \text{tumor weight of treated}}{\text{tumor weight of control group}} \times 100$$

20

TABLE 13

Effect of I-10 on the Growth of Chondrosarcoma in Rats

Group	Animals		Body Weight		Tumor Weight (grams)	Inhibition rate (%)
	initial	final	initial	final		
Control	10	10	95.4	251.0	14.2	
Treated # 5 mg/kg	10	10	97.8	258.4	12.1	14.8
10 mg/kg	10	9	98.2	248.0	12.8	9.8
20 mg/kg	10	9	97.4	253.1	8.2	42.2*

P.O. Administration -- * $P < 0.05$

TABLE 14

Effect of II-A on the Growth of Chondrosarcoma in Rats

Group	Animals		Body Weight		Tumor Weight (grams)	Inhibition rate (%)
	initial	final	initial	final		
Control	10	10	70.6	218.8	10.7	
2 mg/kg x10	10	9	74.1	130.6	5.4	49.5
5 mg/kg x10	10	9	71.7	129.2	4.0	62.6

Example 23: Effect of Retinoids on Cancer Cell
Differentiation and Cancer Cell Survival

The protocols of Examples 14 and 21 were used, for the NBT and MTT assays, respectively.

5

Table 15

Effect of Coumarin retinoids on HL-60 cells

10

Compound	NBT ED ₅₀	MTT (mol/L)
I-28	$> 10^{-5}$	$> 10^{-5}$
I-29	$> 10^{-5}$	1.9×10^{-6}
I-30	$> 10^{-5}$	$> 10^{-5}$
I-31	$> 10^{-5}$	$> 10^{-5}$
I-32	1.3×10^{-5}	1.1×10^{-5}
I-33	$> 10^{-5}$	3.2×10^{-6}
I-34	$> 10^{-5}$	$> 10^{-5}$
I-35	6.7×10^{-6}	2.6×10^{-6}
I-36	$> 10^{-5}$	3.2×10^{-6}

15

Table 16

Effect of purine retinoid compounds on HL-60, HCT-8, A2780, KB and BEL 7402 cell lines

20

Comp.	NBT (mol/L) HL-60	MTT ED ₅₀ (mol/L)			
		HCT-8	A2780	KB	Bel7402
II-D	$> 10^{-5}$	4×10^{-6}	$> 10^{-5}$	$> 10^{-5}$	5×10^{-6}
II-F	$> 10^{-5}$	$> 10^{-5}$	$> 10^{-5}$	$> 10^{-5}$	$> 10^{-5}$

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

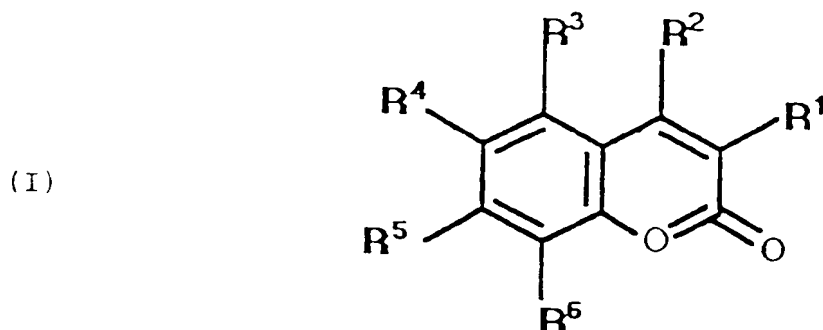
The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range

of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that

5 the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

WHAT IS CLAIMED IS:

1. A compound according to formula (I):



wherein R¹ = H, C₁-C₁₈ alkyl, or COR⁷, where R⁷ = H, OH, C₁-C₁₈ alkoxy, or NHR⁸, where R⁸ = H, C₁-C₁₈ alkyl, unsubstituted phenyl, or phenyl substituted with OH, COOH, OCH₃, or NO₂; R² = H, OH, C₁-C₁₈ alkyl, or OR, where R=retinoyl; R³ = H, CH₃, COR⁷, OR, or CH₂OR, where R and R⁷ are defined above; R⁴ = H, halogen, OH, C₁-C₁₈ alkyl, COR⁷, or OR, where R and R⁷ are defined above; R⁵ = H or OR, where R is defined above; and R⁶ = H, CH₃, COR⁷, or OR, where R and R⁷ are defined above; wherein at least one of R²-R⁶ contains a retinoyl group.

2. A compound according to claim 1, wherein R¹=H or COR⁷, where R⁷ is CH₃, C₂H₅, NH₂, or OH.

3. A compound according to claim 1, wherein R²=H, OR, or CH₃, where R = retinoyl.

4. A compound according to claim 1, wherein R³=H, CH₃, OR, CH₂OR, or COR⁷, where R=retinoyl and R⁷=OH, C₂H₅ or OC₁₂H₂₅.

5. A compound according to claim 1, wherein R⁴=H, Cl, COOH, C₂H₅, C₆H₁₃, COCH₃, or COC₆H₅.

6. A compound according to claim 1, wherein R⁵=H, or OR, where R=retinoyl.

7. A compound according to claim 1, wherein R⁶=H, COOH, COCH₃, CH₃, or OR, where R=retinoyl.

8. A compound according to claim 1, wherein said compound is selected from the group consisting of (I-1), (I-2), (I-3), (I-4), (I-5), (I-6), (I-7), (I-8), (I-9), (I-10), (I-11), (I-12), (I-13), (I-14), (I-15), (I-16), (I-17), (I-18), (I-19), (I-20), (I-21), (I-22), (I-23),

(I-24), (I-25), (I-26), (I-27), (I-28), (I-29), (I-30),
(I-31), (I-32), (I-33), (I-34), (I-35), (I-36), (I-37),
(I-38) and isomers thereof.

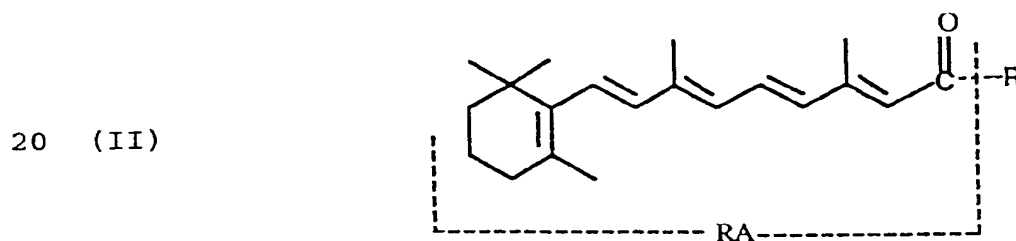
9. A compound according to claim 1, wherein $R^1 = \text{COCH}_3$,
5 $R^2 = R^3 = R^4 = \text{H}$, and $R^5 = R^6 = \text{OR}$, where $R = \text{retinoyl}$.

10. A pharmaceutical composition comprising a
compound according to claim 1, and a pharmaceutically
acceptable carrier.

11. A pharmaceutical composition according to claim
10, further comprising a drug selected from an anti-cancer
compound or composition or an immunomodulator.

12. A method for inhibiting precancer or
cancerrelated pathology in cells or tissue of a mammal,
comprising the step of administering an effective precancer or
15 cancer inhibiting amount of a compound according to claim 1.

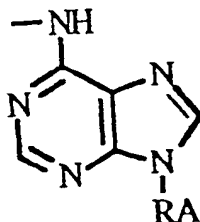
13. A compound according to formula (II):



wherein R is a purine derivative.

25 14. A compound according to claim 13, wherein said
compound is selected from the group consisting of (II-A),
(II-B), (II-C), (II-D), (II-E), (II-F), (II-G), (IIH), (II-I),
(II-J), (II-K), (II-L), and isomers thereof.

30 15. A compound according to claim 13, wherein



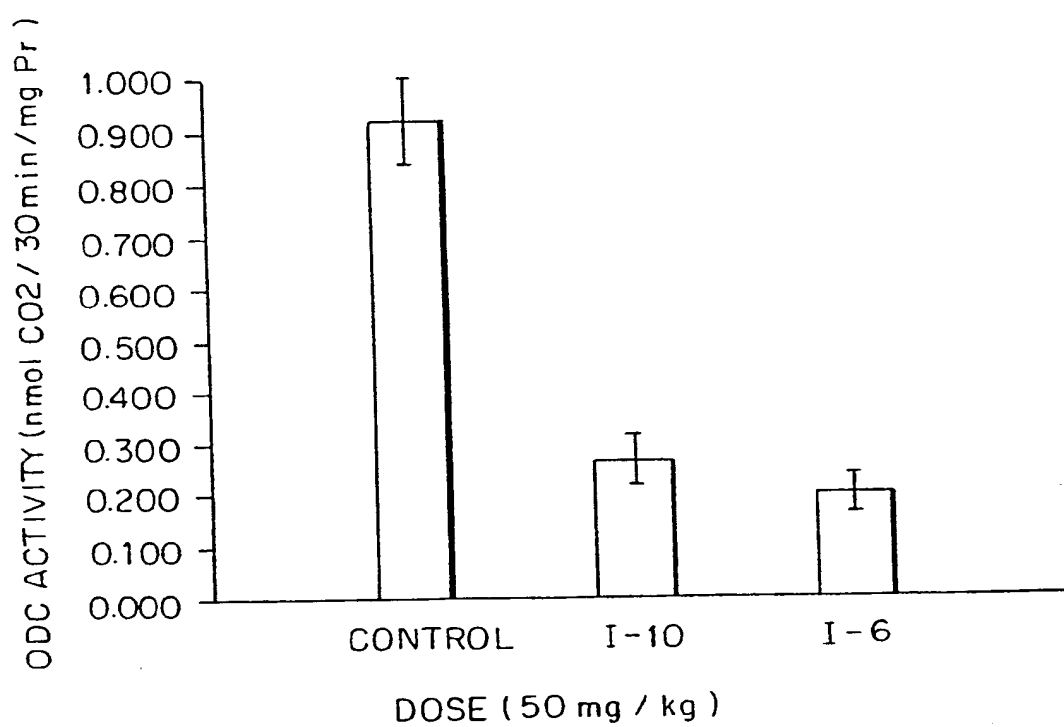
35 16. A pharmaceutical composition comprising a
compound according to claim 13, and a pharmaceutically
acceptable carrier.

17. A pharmaceutical composition according to claim 16, further comprising a drug selected from an anti-cancer compound or composition or an immunomodulator.

18. A method for inhibiting precancer or cancer-
5 related pathology in cells or tissue of a mammal, comprising the step of administering an effective precancer or cancer inhibiting amount of a pharmaceutical composition according to claim 16.

1 / 1

FIG. 1



INTERNATIONAL SEARCH REPORT

Inter- national Application No
PCT/US 96/10507

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D311/16 C07D311/14 C07D473/00 C07D473/18 C07D473/24
C07D473/34 A61K31/52 A61K31/37

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 96, no. 30, 1982 Columbus, Ohio, US; abstract no. 123037g, XU, SHIPING ET AL.: "STUDIES ON ANTITUMOR AGENTS. SYNTHESIS OF DERIVATIVES OF RETINOIC ACID." page 723; column 2; XP002013792 see abstract & YAOXUE XUEBAO, vol. 16, no. 9, 1981, CHINA, pages 678-686, -----	1-7, 10-12
X		1-7, 10-12

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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- *O* document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search

19 September 1996

Date of mailing of the international search report

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